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<th>Instructions for use of SEASONAL CHANGES IN BODY SIZE AND OIL SAC VOLUME OF THREE PLANKTONIC COPEPODS, PARACALANUS PARVUS (CLAUS, 1863), PSEUDOCALANUS NEWMANI FROST, 1989 AND OITHONA SIMILIS CLAUS, 1866, IN A TEMPERATE EMBAYMENT: WHAT CONTROLS THEIR SEASONALITY?</th>
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<td>Author(s)</td>
<td>Arima, Daichi; Yamaguchi, Atsushi; Abe, Yoshiyuki; Matsuno, Kohei; Saito, Rui; Asami, Hiroki; Shimada, Hiroshi; Imai, Ichiro</td>
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SEASONAL CHANGES IN BODY SIZE AND OIL SAC VOLUME OF THE THREE PLANKTONIC COPEPODS, *PARACALANUS PARVUS* (CLAUS, 1863), *PSEUDOCALANUS NEWMANI* (FROST, 1989) AND *OITHONA SIMILIS* (CLAUS, 1866), IN A TEMPERATE EMBAYMENT: WHAT CONTROLS THEIR SEASONALITY?

BY

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Short title: SEASONALITY IN COPEPOD BODY SIZE AND OIL SAC VOLUME
ABSTRACT

Seasonal changes in body size (prosome length: PL) and oil sac volume (OSV) of the three most numerically abundant copepods in Ishikari Bay, northern Sea of Japan, *Paracalanus parvus* (Claus, 1863), *Pseudocalanus newmani* (Frost, 1989) and *Oithona similis* (Claus, 1866), were studied using monthly samples collected through vertical hauls of a 100-μm mesh NORPAC net from March, 2001 to May, 2002. Seasonal changes in PL were common for the three species and were more pronounced during a cold spring. PL was negatively correlated with temperature, and this relationship was described well using the Bělehrádek equation. Seasonal changes in OSV exhibited a species-specific pattern: i.e., OSV was greater during a warm summer for *P. parvus* and was greater during a cold spring for *P. newmani* and *O. similis*. The OSV peak period corresponded with the optimal thermal season of each species. The relative OSV to prosome volume of the small copepods (0.6-0.8%) was substantially lower than that of the large copepods (20-32%). These facts suggest that the oil sac of small copepods is not used for overwintering or diapauses or during periods of food scarcity but is instead used as the primary energy source for reproduction, which occurs during the optimum thermal season of each species.
INTRODUCTION

For planktonic copepods, body size is expressed as the total length or the prosome length (PL), and the oil sac is quantified as the oil sac volume (OSV). These are two of the easiest to measure variables for assessing population structure. Regarding PL, the total egg volume of Pseudocalanus species can be determined from the female PL alone and appears to be unaffected by food supply (Corkett & McLaren, 1969). For the small copepods (five species belonging to four genera) that predominate at Helgoland Roads, southern North Sea, significant correlations were only found between clutch size or egg production rate and PL, and PL was also correlated with temperature (Halsband & Hirche, 2001). Therefore, researchers concluded that water temperature controlled copepod egg production by affecting PL at a certain threshold of food availability (Halsband & Hirche, 2001). The relationship between PL and temperature is described well by the Bělehrádek equation (Corkett & McLaren, 1969, 1978). Egg diameter and clutch size were also strongly correlated with PL in cyclopoid copepods (Dvoretsky & Dvoretsky, 2009). For copepod populations, PL is important for the identification of generations and the estimation of secondary production (Renz et al., 2007, 2008); thus, PL is useful for the population structure analysis of planktonic copepods.
Regarding OSV, the accumulation and utilisation of storage lipids is necessary for reproduction, growth, coping with food scarcity and diapause (Lee et al., 2006). Wax esters and triacylglycerols are the lipids commonly stored by copepods. Lipid reserves are known to vary with the seasons (Kattner & Krause, 1989; Norrbin et al., 1990), which is also a common pattern for deep-sea copepods (Auel & Hagen, 2005). Few studies, however, have attempted to assess seasonal changes in both PL and OSV within the same copepod population (cf. Pepin & Head, 2009). To clarify how seasonal changes affect PL and OSV in copepod populations, they both need to be analysed in the same copepod population.

In the present study, we observed seasonal changes in the PL, OSV and population structure of three sympatric small copepods: Paracalanus parvus (Claus, 1863), Pseudocalanus newmani (Frost, 1989) and Oithona similis (Claus, 1866). Over a period of one year, we collected seasonal plankton samples from a small temperate bay using a fine-mesh (100 µm) net. In this study, we discuss the mechanisms governing the seasonal changes in PL and OSV by comparing those indices in different species over the course of one year (P. parvus vs. P. newmani vs. O. similis).

MATERIAL AND METHODS
Field sampling

One or two month interval samplings were conducted at a single station (43°20’N 141°10’E, 40 m bottom depth) in Ishikari Bay from March 12, 2001 to May 14, 2002 (n = 12) (fig. 1A, B). Zooplankton samples were collected using vertical hauls of a NORPAC net (45 cm mouth diameter, 100 μm mesh; Motoda, 1957) from a 35 m depth to the surface. A flow meter (Rigosha Co. Ltd.) mounted on the net ring allowed the filtered water volume to be quantified. The zooplankton samples were immediately preserved with 5% formaldehyde-seawater.

Water temperature and salinity were measured by CTD (Seabird SBE911) casts during each sampling. In addition, 300-ml seawater samples were collected at 5- or 10-m intervals using a Nansen bottle or bucket at the surface and were filtered through GF/F filters. The filters were then immersed in 90% acetone and subjected to dark conditions for one day, and the chlorophyll a (Chl. a) concentration was measured using a fluorometer (Turner Model 10AU).

Sample analysis

The zooplankton samples were sorted into the three numerically dominant species (Paracalanus parvus, Pseudocalanus newmani and Oithona similis), and the
numbers of each species were counted under a stereomicroscope. For up to 50 adult females (C6F) of these species per sample, prosome length (PL), prosome width (PW), oil sac length (OSL) and oil sac width (OSW) were measured using an eyepiece micrometre mounted in an inverted microscope that had a precision of 10 μm. Prosome volume (PV, μm$^3$ ind.$^{-1}$) and oil sac volume (OSV, μm$^3$ ind.$^{-1}$) were calculated assuming an ellipsoid shape (Narcy et al., 2009) using the following equations:

\[
PV = \frac{1}{6} \pi PW^2 PL \quad (2)
\]

The presence of an oil sac and the OSV were expressed in three ways: percentage composition of oil sac containing females to total females (%), absolute lipid amount (OSV, μm$^3$ ind.$^{-1}$) and the percentage of PV that was OSV (OSV:PV, %).

The seasonality of PL, OSV and OSV:PV of the three dominant small copepods was tested using an ANOVA with Welch procedure. For the seasonality of the frequency of females with an oil sac, we applied a chi-square test to the monthly data.

RESULTS
Throughout the year, integrated mean salinity (0-35 m) showed little seasonality, with values of approximately 33.5-34.0 PSU, while temperature demonstrated a clear seasonal pattern: lower (<5°C) during the spring (February to March) and higher (>15°C) during the summer (July to October) (fig. 1C). Chl. a was highest (3.87 mg m⁻³) from March to April.

**Paracalanus parvus** (Claus, 1863)

The abundance of *Paracalanus parvus* was greater in July and September, with the peak abundance (9,392 ind. m⁻³) in July, and *Paracalanus parvus* was not present in the samples taken from immediately after the peak abundance until the following spring (March to April 2001) (fig. 2A). All of the copepodid stages were observed from June to March. The mean PLs of C6F ranged from 534 to 631 μm. The PL showed clear seasonal changes, being longer in the spring and shorter in the summer (p<0.001, ANOVA with Welch procedure). The percentage of females with an oil sac was greater during the summer (July to October 2001) (p<0.001, chi-square test). OSV and OSV:PV showed parallel seasonal changes. Both OSV and OSV:PV were greater during the summer (July to September 2001) (p<0.001, ANOVA).
**Pseudocalanus newmani** (Frost, 1989)

In contrast to *Paracalanus parvus*, *Pseudocalanus newmani* was not present in the samples taken during the summer (September and October 2001) (fig. 2B). The abundance of *P. newmani* peaked at 3,861 ind. m$^{-3}$ in May, 2001. From February to May, the early copepodid stages (C1-C3) were abundant. The mean PLs of this species ranged from 669 to 991 μm, and the PL was longer during the spring and shorter during the summer (p<0.001, ANOVA with Welch procedure). The percentage of females with an oil sac was greatest during the spring (March to April) (p<0.001, chi-square test). OSV and OSV:PV also showed parallel seasonal changes for this species, and their seasonality was greatly different from that of *P. parvus*, mentioned above. For *P. newmani*, the highest OSV and OSV:PV was observed during the spring (March-May) (p<0.001, ANOVA).

**Oithona similis** (Claus, 1866)

*Oithona similis* was present in the samples taken throughout the year. The abundance of *O. similis* peaked in April (12,110 ind. m$^{-3}$) (fig. 2C). The early copepodid stages (C1-C3) were abundant from winter to spring. The mean PLs of *O. similis* ranged from 377 to 439 μm and were shorter during the summer and longer
during the spring (p<0.001, ANOVA with Welch procedure). The percentage of females with an oil sac was greatest during the spring (February to May) (p<0.001, chi-square test). This species also showed parallel seasonal variations for OSV and OSV:PV. A high lipid accumulation was observed during the spring, and a low lipid accumulation was observed from June to December (p<0.001, ANOVA).

DISCUSSION

The three species analysed here showed distinct seasonal occurrences: *Paracalanus parvus* was absent during the cold spring, *Pseudocalanus newmani* was absent during the warm summer and *Oithona similis* was present year-round with its greatest abundance being in the cold spring. From these seasonal occurrence patterns, the thermal habitat of each species was determined. *P. parvus* is classified as a warm-water species, and *P. newmani* and *O. similis* are classified as cold-water species. The thermal characteristics of each species in this study corresponded well with previously published results concerning these species in various locations in the Sea of Japan (Dolganova et al., 1999; Iguchi et al., 1999; Takahashi & Hirakawa, 2001).

Prosome length (PL)
Although the optimum thermal condition was different for each species, the PLs of the three species showed a common seasonal pattern (longer in the cold spring, fig. 2A-C). Seasonal changes in copepod PL have been described by numerous studies. The PL of adults is the result of the increments of successive moults during development (Mauchline, 1998 and references therein). The length achieved during an intermoult period, however, bears some relation to the duration of the period, and the duration of the period is strongly influenced by the environmental temperature. The relationship of length with environmental temperature is linear in females (Deevey, 1960). However, Liang & Uye (1996) and Liang et al. (1996) found that the Bělehrádek equation describes the relationship of mean body size with temperature at the time of sampling of each naupliar and copepodid of Acartia omorii (Bradford, 1976) and Centropages abdominalis (Sato, 1913).

For the three species in this study, the relationship between PL and temperature could be expressed by both the Bělehrádek equation and a linear equation (table I, fig. 3A). As is common for these three species, the coefficients of determination ($r^2$) were higher for the Bělehrádek equation than the linear equation. This suggests that the Bělehrádek equation is a superior for measuring the relationship between PL and temperature. Because the relationship between copepod development time (=
intermoult period) and temperature is described well by the Bělehrádek equation (cf. Corkett & McLaren, 1978), PL may reflect the environmental temperature during the copepods’ intermoult periods.

As an environmental parameter, the relationship between PL and Chl. a was also tested (fig. 3C). However, no significant relationship between PL and Chl. a was observed for any of the three species. This may be due partly to the location of this study: a eutrophic neritic embayment, which is expected to be less food-limited throughout the year. Under such conditions, the intermoult period of copepods might be governed by temperature exclusively (Corkett & McLaren, 1969; Halsband & Hirche, 2001).

Oil sac volume (OSV)

Seasonality in OSV in this study varied between species: i.e., a greater OSV was observed during the warm summer for *P. parvus*, and the same result was observed during the cold spring for *P. newmani* and *O. similis* (fig. 2A-C). The year-round occurrence of *O. similis* and its greater abundance during the cold spring season suggest that this species is adapted to cold-water conditions (Dolganova et al., 1999; Iguchi et al., 1999; Takahashi & Hirakawa, 2001). Considering *P. parvus* as a warm-water
species and *P. newmani* and *O. similis* as cold-water species, the seasonal changes in OSV corresponded with the optimal temperature seasons of each species. Thus, OSV was greater during a high temperature season for the warm-water species *P. parvus*, and OSV was greater during a low temperature season for the cold-water species *P. newmani* and *O. similis* (fig. 3B).

Researchers argue whether the oil sac is used for energy preservation for reproduction, food scarcity, ontogeny or diapause (see Lee et al., 2006 and references therein). The species-specific seasonality of OSV observed in this study did not take into account oil sac utilisation for diapause or overwintering. A lower correlation between OSV and food (Chl. a) (fig. 3D) also eliminates the possibility of oil sac utilisation as an adaptation to food scarcity. The species-specific seasonality of OSV for the three species studied here suggests that the OSV is large during the reproduction period of these species. It should be noted that OSV may decrease within an individual during reproduction, if it is measured using a short temporal scale. However, these smaller temporal scale changes within individuals may difficult to evaluate using our sampling design (one month intervals). To overcome this problem, individuals need to be incubated in a laboratory setting in the future.

Lischka & Hagen (2007) reported that *O. similis* in Svalbard used wax esters to
fuel gonad maturation and egg production for reproduction in June and that *O. similis* reproduction in August/September co-occurred with the accumulation of new depot lipids. *Pseudocalanus elongatus* (Boeck, 1865) was also reported to use lipid reserves for reproduction in the Central Baltic Sea (Peters et al., 2006).

It should be noted that the OSV:PV of the small copepods in this study was substantially lower than that of the large-sized copepods. The oil sac in *Calanus euxinus* (Hülsemann, 1991) composes 25-32% of its body volume (Vinogradov et al., 1992). The oil sac of *Calanus finmarchicus* (Gunnerus, 1770) composes approximately 20% of its body volume (Plourde & Runge, 1993), and in *Metridia pacifica* (Brodsky, 1950), when the oil sac is at its largest size, it composes 20% of its body volume (Hirakawa & Imamura, 1993). For this study, the maximum OSV:PV values were 0.6-0.8% (fig. 2A-C). This discrepancy in OSV:PV between small and large-sized copepods suggests that the function of lipid reserves may vary between the two species groups. For small-sized copepods, because they have less lipid reserves, they are not used for overwintering, diapause or during periods of food scarcity, but are instead used primarily for reproduction. Because no correlation between OSV and food was determined (Chl. a, fig. 3D), and the high OSV period corresponded with the optimum thermal seasons of each species (fig. 2A-C), we propose that small copepods in
temperate embayments primarily use stored lipids for reproduction.

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CAPTIONS

Fig. 1.  A, Location of Ishikari Bay in the Sea of Japan; B, sampling station (star) in Ishikari Bay.  C, Seasonal changes in depth integrated mean (0-35 m) temperature, salinity and chlorophyll a (Chl. a) in Ishikari Bay during March, 2001 to May, 2002. Depth contours (10, 30, 50, 100, 200 and 600) are superimposed in B.

Fig. 2. Seasonal changes in abundance and population structure (upper), prosome length (PL) (upper middle), percentage of females with oil sac (lower middle), oil sac volume (OSV) and OSV:PV percentage (lower) of A, Paracalanus parvus (Claus, 1863); B, Pseudocalanus newmani (Frost, 1989); C, Oithona similis (Claus, 1866) in Ishikari Bay from March, 2001 to May, 2002. Symbols and bars of PL, OSV and OSV:PV indicate means and standard deviations, respectively.

Fig. 3. In adult females, relationship between A, mean prosome length (PL) and temperature; B, mean oil sac volume (OSV) and temperature; C, mean PL and chlorophyll a (Chl. a); D, mean OSV and Chl. a for Paracalanus parvus (Claus, 1863) (left), Pseudocalanus newmani (Frost, 1989) (middle) and Oithona similis (Claus, 1866) (right) in Ishikari Bay during March, 2001 to May, 2002. For significant relationships, regression lines (Bělehrádek equations) are shown.
**: p<0.01, ***: p<0.001, (ns): not significant.
Table I. Equation between prosome length (PL: μm) or oil sac volume (OSV: x 10³ μm³) and temperature (T: °C) for *Paracalanus parvus* (Claus, 1863), *Pseudocalanus newmani* Frost, 1989 and *Oithona similis* Claus, 1866 in Ishikari Bay. For regressions, two equations (Bělehrádek and linear equations) were applied. \( r^2 \): coefficients of determination. *: p < 0.05, **: p < 0.01, ***: p < 0.001, ns: not significant.

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<td><strong>Bělehrádek equation</strong></td>
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<td>PL= 5.32 (T + 254)₁²₀⁵</td>
<td>0.263***</td>
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<tr>
<td>PL= 2.88 (T + 46.0)₁²₀⁵</td>
<td>0.600***</td>
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<tr>
<td>PL= 3.03 (T + 228)₁²₀⁵</td>
<td>0.579***</td>
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Fig. 2. Arima et al.
Fig. 3. Arima et al.